

# LabLink

Laboratory Information from the Michigan Department of Community Health Bureau of Laboratories

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# The Bioterrorism Laboratory Response Network

Frances Pouch Downes, Dr. P.H. Director of Laboratories

On November 2, 1999 the Michigan Department of Community Health laboratories hosted a conference to introduce the medical laboratory community to the Bioterrorism Laboratory Response Network (LRN). The meeting was presented in cooperation with our partners at the Centers for Disease Control and Prevention and the National Laboratory Training Network. The conference covered the need for the development of a coordinated response, clinical aspects of likely bioterrorist agents and role of the clinical microbiology laboratory in detecting and responding appropriately to identification of a bioterrorist agent. This issue of LabLink is devoted to issues of which all clinical laboratory managers and staff should be aware.

What is bioterrorism? Bioterrorism is the intentional use of microbial agents or their toxins or chemical agents to do harm to individuals or populations. A bioterrorist attack may be politically motivated or motivated by personal reasons.

Bioterrorist activities can present either as an announced attack, where the attacker identifies the agent used and possibly other information about the attacker, or as a unannounced or covert attack. While victims of the announced attack will be readily identifiable, victims of the unannounced attack may not be identified until they present for medical care. Emergency departments, urgent care clinics and medical laboratories, may then be the first to recognize the results of the attack.

The agents most likely to be used are those that are not endemic or typically present in Michigan. Practitioners will need to familiarize themselves with the clinical presentation of these agents. The clinical microbiology laboratory that has been designated Level A lab will need to have procedures in place to rule out and refer on suspect isolates (see figure 1 on page 2). In the LRN any clinical microbiology laboratory performing such basic testing as blood culture may identify a suspect bioterrorism agent.

Once a suspected agent has been identified the closest Level B laboratory should be contacted. The Level B laboratories in Michigan are six public health laboratories with the capacity to perform confirmatory testing (also figure 1). These labs will the confirm the initial identification (rule in) or if testing is inconclusive the isolate will be sent (refer on) to a Level C laboratory. In Michigan the only Level C laboratory will be the state public health laboratory in Lansing.

Announced attacks will only be addressed with an opposite flow. All specimens collected during the announced attack will be directly transported by law enforcement to the Level C laboratory or Level D facility.

What do laboratory managers need to do now? Review safety procedures to determine if the laboratory could safety handle an initial culture and preliminary identification, review testing procedures to determine if they are consistent with recommended identification schemes, assure that appropriate packaging is on hand, and contact the local health department and law enforcement agency to become integrated into the local response planning.

The LRN provides a solid framework for reporting and responding to any infectious disease. The recognition by the medical community, transfer of information to public health agencies and the implementation of an appropriate response are key activities to address other potentially infectious disease outbreaks. Collaborations formed during bioterrrorism response plan development will be equally valuable in strengthening our surveillance and control of endemic and emerging infectious diseases.

### Detection of Biologic Agents of Bioterrorism

Barbara Robinson-Dunn, Ph.D. Microbiology Section

The thought that bioterrorism may occur in Michigan is frightening. Viewers of the Nightline series on a fictional account of a bioterrorist attack saw a projection of the terrible after-effects following such an event. Preparation and planning are necessary so that if an attack does occur, microbiologists will be able to rapidly identify the probable agent(s). The most likely agents of a bioterrorist attack are known as the priority biologic agents and include Bacillus anthracis, Francisella tularensis, Yersinia pestis, Clostridium botulinum, Brucella species and smallpox virus.

Since they are on the front-lines, Level A laboratories will have the responsibility to rule out the priority biologic agents. Any suspected agents will be forwarded to the Level B laboratories for identification and initial characterization. This might include DFA, latex agglutination testing, phage typing and perhaps antimicrobial susceptibility testing. Cultures will be forwarded to MDCH for more complete analysis which might include animal inoculations for botulism, subtyping, molecular analyses or additional susceptibility testing. All characterized agents will be forwarded to CDC as will any suspect specimens for smallpox since that agent must be handled only in a biosafety level 4 facility.

Training of the Level A laboratories has begun and will soon be followed by training aimed at the Level B and Level C laboratories. A project coordinator for the biologic agents component of the project will be hired. This person will work closely with laboratories licensed to perform microbiology in Michigan to ensure that laboratories will be ready should this frightening event occur. Should you have any questions about the laboratory detection of the priority biologic agents, please contact the Division of Infectious Diseases at (517) 335-8067.

LEVIL D: CDC

LEVEL C: Typing Labs, Public Health Labs

LEVEL A: Chaical Labs

LEVEL A: Chaical Labs

### Chemical Terrorism

Jacqueline S. Scott, D.V.M., Ph.D. Division of Chemistry & Toxicology

Experts on terrorism agree that toxic industrial chemicals can cause mass casualties and require little expertise or sophisticated methods. Toxic industrial chemicals can be bought on the commercial market or stolen, thus avoiding the need to manufacture them. Chlorine, phosgene, and hydrogen cyanide are a few of these chemicals. Unlike toxic industrial chemicals most G and V chemical nerve agents are technically challenging for terrorists to acquire, manufacture and produce. Examples of the G-series are tabun (GA), sarin (GB), and soman (GD). VX is an example of a V-series nerve agents. Developing nerve agents requires synthesis of multiple precursor chemicals. Although tabun production is relatively easy, containment of a highly toxic gas (hydrogen cyanide) is a technical challenge. Production of sarin, soman, and VX requires the use of high temperatures and generates corrosive and dangerous by products. Blister chemical agents such as sulfur mustard and nitrogen mustard can be manufactured with ease or with only moderate difficulty but buying the precursor chemicals is difficult.

Chemical agents need to be in vapor or aerosol form to cause optimal inhalation exposure to cause an effect. Vapors and aerosols remain suspended in the air and are readily inhaled deep into the lungs. Another method is to spray large droplets or liquid for skin penetration. A chemical agent could be disseminated by explosive or mechanical delivery. Terrorists could disseminate chemical agents using simple containers such as glass bottle with commercial sprayers attached to them or fire extinguishers.

The MDCH Division of Chemistry and Toxicology is one of four state laboratories to be awarded a cooperative agreement with the CDC for laboratory analysis of potential chemical terrorist agents. The analyses will be performed on mass spectrometers, gas chromatographs and high performance liquid chromatographs as a screening panel developed by CDC. The MDCH laboratory is awaiting arrival of the equipment and will begin the first staff training session at CDC in December.

figure 1

# Bartonella Testing Now Available at MDCH

Patty Clark, M.P.H. Viral Serology/Viral Isolation Unit

Cat-scratch disease (CSD) is said to be the leading cause of chronic regional adenopathy in children in the United States. This suggests that CSD is among the most common zoonotic diseases in the U.S. It is now understood that CSD, a syndrome recognized for at least the last 50 years, is caused by Bartonella henselae. A variety of recently described syndromes in immune-impaired patients (e.g., bacillary angiomatosis [BA]) are also caused by either B. henselae or B. quintana. B. quintana has not been associated with CSD. Both B. henselae and B. quintana are fastidious, relatively slow-growing bacteria, that may not be cultivated using standard operating protocols found in many clinical laboratories. In part due to the difficulty in detecting growth within a reasonably short period, laboratory diagnosis for Bartonella spp. currently depends primarily on serology.

An indirect immunofluorescence assay (IFA) for the detection of *Bartonella* was developed at the Centers for Disease Control and Prevention (CDC). IFAs have been used for many years in the diagnostic laboratory. This technique provides a relatively simple method for detecting antibodies to a wide variety of pathogens. Because only a small amount of antigen is needed for each test, the IFA provides an economical serologic assay.

Most patients have elevated antibody titers at the time of their first visit to a health care provider. However, if titers are low or nonexistent, and the patient was seen within the first two to three weeks of onset of signs, a second serum specimen should be evaluated for more complete laboratory diagnosis. High-titered, anti-B. henselae antibodies for persons with CSD appear to typically decline within one (1) year after onset of symptoms.

MDCH now offers IFA testing for both Bartonella henselae and Bartonella quintana. Serum (1 ml is optimum) must be submitted in a plastic tube. To order, use the "Other" line in the Viral Serology box on the red and white FB200 Virology Test Request form. Tubes and forms are available by faxing Ron Dietz at 517-335-9039 and requesting Unit #8. Questions about Bartonella testing can be directed to the viral serology lab at 517-335-8102.

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### New Employees, Transfers and Promotions

MDCH and the Bureau of Laboratories would like to welcome five new employees. Pat Reilley has joined newborn screening. Tom Williams and Nancy Bogart are now working in the DASH unit. Williams came to MDCH from BioPort and Bogart transferred from the Department of Management and Budget. Denise Nightengale has joined the bureau and will divide her time between virology, microbiology and epidemiology. Jolene Vanneste is currently working as a microbiologist in the microbiology section. Vanneste had worked in microbiology, virology and epidemiology as an employee of the Michigan Public Health Institute.

Anuradha Patel be transferring from the microbiology section to newborn screening.

Anne Clark of newborn screening has been promoted from the position of laboratory technician to the position of laboratory scientist.



### MDCH INITIATES HIV-1/HIV2 SERUM TESTING

Deborah Stephens, MT (ASCP) HIV Unit

Effective October 4, 1999, MDCH, in cooperation with the regional laboratories of Kent County and Detroit health departments, replaced the current serum HIV-1 screening test. The new, more sensitive test also incorporates HIV-2 testing. The HIV1/HIV-2 screening test is based on synthetic peptides derived from highly conserved, immunodominant regions of gene products for both HIV-1 and HIV-2. It offers improved performance over the currently licensed HIV-1/HIV-2 whole viral lysate tests. This new test has the additional advantage detecting all nine known HIV-1 group O's, divergent strains including Group M subtypes (A-H and Thai-B). The test has a reported sensitivity for both HIV-1 and HIV-2 of 100%, and a specificity of 99.9%. Our in-house evaluation confirmed this performance.

MDCH will continue to use the CDC/APHL testing algorithm (MMWRVol 41, 1992). All specimens which are reactive in the HIV-1/HIV-2 screening EIA test, and non-reactive or indeterminate for HIV-1 Western blot at the regional labs will be transferred to Lansing's MDCH HIV laboratory for HIV-2 EIA testing. Results will be sent back to the regional lab, and reports of the HIV-2 EIA results will be mailed to the original submitter.

The next printing of DCH-0567 (Michigan Regional Laboratories Test Requisition) will reflect the change to HIV-1/HIV-2 for serum screening testing. To receive test requisition forms and specimen container orders, phone (517)335-9333. For questions regarding technical/laboratory issues, contact Ken Terpstra at the Kent County Health Department, (616)336-3475, Dr. Aloysius Hanson at the Detroit Regional Laboratory (313)876-4223, or Deborah Stephens at MDCH (517)335-8098.

# HIV-1/HIV-2 Testing Algorithm HIV-1/HIV-2 Peptide EIA RR Neg Report as Negative HIV-1 Western Blot Negative Pos or Indeterminate Report HIV-2 EIA as Positive Neg HIV-2 Confirmatory Report as Negative

### RACCOON RABIES, U.S., OHIO AND MICHIGAN

Patty Clark, M.P.H. Viral Serology/Viral Isolation Unit

A variant of rabies virus associated with raccoons has been present in the southeastern United States since the 1950s and has spread northward into the mid-Atlantic states possibly via the translocution of animals. The first mid-Atlantic state to report a case of raccoon rabies was West Virginia in 1977. Raccoon rabies has been spreading outward ever since. Cases have been reported in Virginia (1978), Maryland (1981), the District of Columbia and Pennsylvania (1982), Delaware (1987), New Jersey (1989), New York (1990), Connecticut and North Carolina (1991), Massachusetts and New Hampshire (1992), Rhode Island, Vermont and Maine (1994), and Ohio (1996). Although westward progression has been slowed by geographic barriers (the Great Lakes, Appalachian Mountains, Ohio River), once the raccoon variant becomes established in the Ohio Valley, it may spread more rapidly across the Midwest.

In May 1996, Ohio reported its first indigenous case of raccoon rabies, approximately three miles from the Ohio-Pennsylvania border. Although no other cases were detected in 1996, active surveillance identified several in early 1997. These additional cases sparked a mass oral vaccination program targeting raccoons in affected counties. The protocol for oral vaccination was developed in consultation with CDC. Beginning in mid-1997, fishmeal vaccine-laden baits were airdropped or hand-distributed in target counties. Each bait packet contained rabies virus vaccine shown to be effective in raccoons and harmless to other wildlife, domestic animals, and humans. Traditional rabies control measures were also emphasized including vaccination of pets, postexposure prophylaxis (PEP) of people exposed to rabid animals and public education.

There have been no documented human rabies cases in the U.S. associated with the raccoon rabies variant. Possible explanations for this are 1) diligence in administering PEP to individuals bitten by raccoons, and 2) domestic animal rabies vaccination programs have provided a barrier to human infection.

The costs associated with rabies control and prevention in affected states have increased dramatically. The primary cost increase resulted from an increased number of PEP regimens administered at an average cost (in 1994) of \$1,500 per patient. In Connecticut, the estimated number of persons receiving PEP increased from 41 prior to the introduction of the raccoon rabies virus to 887 during the first nine months post-statewide spread of the raccoon variant. Additional increases in cost result from active surveillance and an increase in the number of animals tested.

This past summer Michigan initiated active surveillance of the raccoon population. Because of resource limitations, surveillance was limited to five southeastern priority counties: Hillsdale, Lenewee, Monroe, Washtenaw and Wayne. Roadkill raccoons and those with obvious neurological impairment were identified

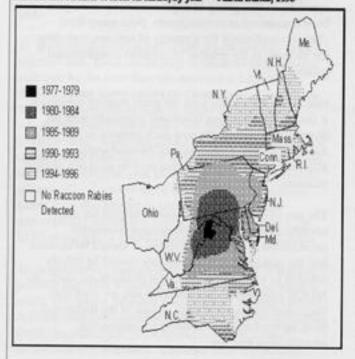
by Animal Control and Protection Shelter personnel, Lenewee County Health Department personnel, and DNR and conservation officers. Carcasses were tagged with date of collection; county, township, and nearest intersection or mile marker; collector's name and phone number; and age and sex of animal. Specimens were prepared for testing and deposited at one of six collection sites throughout the area to be picked up on a weekly basis by Michigan Department of Agriculture (MDA) or Michigan Department of Community Health (MDCH) employees. Specimens were then taken to the MDCH rabies laboratory for testing. The surveillance program started mid-June and went through the end of October. As of October 5, MDCH had tested 75 raccoons, all of which were negative for rabies virus.

MDCH expects to expand this surveillance initiative well into the future. Next year it is planned to increase surveillance to include the lower two tiers of counties in the state. The goal of the surveillance program is to develop disease control plans for human and wildlife populations.

#### References

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- CDC/NCID. Oral vaccination campaign launched to control raccoon rabies epizootic in Ohio. Focus on Viral and Rickettsial Diseases, Volume 6, No. 5, Sept.-Oct., 1997.

PIDURE 1. Detection of mecoon mbies, by year — United States, 1996



### SAGINAW COUNTY REGIONAL LABORATORY

### Tamara Theisen Associate Laboratory Director

In the last issue of LabLink, Kalamazoo County was highlighted as one of the administrative sites. The conception of the Michigan regional laboratory system was also discussed. This issue showcases Saginaw County Department of Public Health, Laboratory Services Division. In the forthcoming issue the remaining three counties will be highlighted.

In 1990, the Michigan regional laboratory system was born and the Saginaw County Department of Public Health (Region II) was named one of five administrative sites. These five sites umbrella several neighboring counties, helping them meet CLIA's guidelines.

The following counties embody Region II: Alcona, Arenac, Clare, Gladwin, Huron, Iosco, Isabella, Ogemaw, Oscoda, Osceola, Roscommon, Saginaw, Sanilac and Tuscola. The laboratory director is Dr. William Sottile from MDCH and the technical consultant is Tammy Theisen from Saginaw County. The laboratory is a division of the Saginaw County Department of Public Health which is under the leadership of Donavon Orth, MPH, RS, Health Officer. Two ASCP medical technologists, a laboratory assistant and the associate laboratory director comprise the laboratory staff in Saginaw County.

Being an administrative hub carries the responsibilities of training, supervising and providing technical expertise. Region II has site coordinator meetings where these vital areas are discussed and training is provided. Saginaw's laboratory provides testing for their region including foodborne outbreaks, stool cultures, chlamydia and gonorrhea analysis. The Saginaw laboratory is licensed to perform water analysis for microbiology and limited chemistries. Toxicology screens are also conducted.

Saginaw County Department of Public Health is proud to be a part of this unique system. Benefits include truly strengthening the quality of test results in neighboring counties and giving non-laboratory staff a better understanding of quality control and quality assurance. The Saginaw laboratory will continue to foster the quality of care in public health by enhancing the quality of testing.

# Quirky Bugs...

### **Enterococci and Other Gram Positive Organisms**

Robert Jacobson, B.S., M(ASCP) Reference Bacteriology Unit

Enterococci can be found in soil, food, water, plants, animals and insects. In humans the enterococci inhabit the gastrointestinal tract (GI) and the female genital tract. The prevalence of the different species appears to vary according to the age, diet and other physiologic conditions of the host. Enterococcus fuecalis are the most common bacteria isolated from the GI tract of humans. Enterococcus faecium also appear to be common from this source. Because of its ubiquitous nature, establishing the clinical significance of enterococci requires a great deal of caution. Potentially misleading laboratory reports need to be avoided, especially when making decisions relating to in vitro susceptibility testing.

Enterococci are associated with a variety of infections. Urinary tract infections are the most common with Enterococci implicated in approximately 10% of infections. Intra-abdominal or pelvic wound infections are the next most common. These are usually polymicrobic and the role of enterococci remains controversial. The increased rate of enterococci in wound infections is probably the result of increased antibiotic use and their emerging resistance. Bacteremia is the third most common type of enterococcal infection. These infections are most often found in the elderly with serious underlying conditions and in the immuno compromised patients with prolonged hospitalization and antimicrobial therapy. Endocarditis is a serious enterococcal infection but it is less common than bacteremia. E. faecalis is the most common species isolated in patients with endocarditis, but other species have been implicated.

Appreciation of the role of enterococci as nosocomial pathogens is clearly on the rise. Enterococci are second only to Escherichia coli as nosocomial urinary tract infections and third as agents of nosocomial bacteremia behind Staphylococcus aureus and coagulase-negative Staphylococci. This trend is likely to continue as the population ages and is put at risk of infection with the deterioration of health due to increasing age.

Atypical strains of Enterococcus species such as E. faecium, E. faecalis, and E. gallinarum are sometimes encountered in clinical laboratories and can be very difficult to identify. Non-motile E. gallinarum can be confused with E. faecium which otherwise is biochemically identical. Vancomycin susceptibility may be helpful in this instance. E. gallinarum is intrinsically resistant due to vanC related resistance and will give an MIC in the range of 8-16µg/ml. Resistant E. faecium, with vanA or vanB resistance, will yield an MIC of >32µg/ml. Resistance to ampicillin and aminoglycosides is uncommon among vanC isolates. Because of rare exceptions, the only way to identify some of the atypical strains is with PCR- based technology. Whole-cell protein profile methods seem to be practical for identification purposes.

Along with enterococci, the reference laboratory at MDCH receives many gram positive organisms for identification. Most are irregular gram positive rods and coccobacilli along with some cocci. Some of the members of this group of organisms have in the past been considered as contaminants. Now many have clinical significance for a variety of reasons, including the increase in the number of immuno compromised patients.

One of the difficulties with the gram positive organisms, is the rapidly changing taxonomy especially with the diphtheroids. There are now such genuses as Aureobacterium, Cellulomonas, Dermabacter, Microbacterium and others that may be seen in a clinical situation.

The use of commercial kits can be helpful for the identification of most of the isolates commonly encountered in clinical specimens. It should be stressed that the manufacture's instructions should be strictly adhered to whenever commercial products are used. At MDCH commercial identification kits are used with traditional biochemical tests. Some of the traditional tests, recommended in addition to commercial identification kits are:

Vancomycin and penicillin susceptibility 6.5% NaCl and bile esculin Motility, urea, nitrate, esculin Gram stain morphology in thio broth Catalase activity
CAMP reaction
Lipophilism (use Tween 80 or serum)
Dextrose fermentation or oxidation
Hemolysis on blood agar plates
PYR and LAP tests
Temperature studies (10°C and 45°C)

Most of these tests can be done with materials on hand. Advanced testing procedures used at MDCH include GLC for detection of metabolic byproducts of growth in the presence of glucose and cell wall fatty acids analysis and PFGE for other epidemiological studies.

Other problem isolates received at MDCH on a regular basis include:

- Aerobically growing Actinomyces species. This genus contains several newly described species. The most common isolate is A. israelii which is usually identified well with commercial systems. Some of the other commonly seen isolates are also identified reasonably well with the commercial identification systems but care must be taken to ensure that the algorithm database of the identification kit in use is current. The identification of the less frequently seen isolates should be left to a reference laboratory that equipped to accomplish this task.
- 2) Over decolorized Bacillus species received as gram negative rod. These are usually medium to long straight rods that by the time they are received at MDCH are demonstrating many spores on Gram stain. Some of the Bacillus species do decolorize easily on Gram stain, which may lead to them being worked up as gram negative rods. When struggling with the identification of questionable gram-negative rods, restain culture material from older plates to look for spores. Bacillus species do occasionally sporulate more readily on urea or esculin slants. It is also helpful to perform a Vancomycin susceptibility screen test (using a 30ug disk) and a 3% KOH string test to establish true nature of the organism.
- 3) Streptococcus mutans routinely submitted as a gram positive rod. This organism should be suspected when a small, dry, white, adherent colony grows on blood plates. A Gram stain performed on material from a thioglycollate broth (incubated for 18-24 hours) will give typical streptococcal appearance. Colonies on a 5% sucrose plate will produce water droplet formations in the primary streak area.

Catalase negative gram positive cocci other than the streptococci and enterococci can be another difficult to identify group of organisms. The reference laboratory at MDCH has found that the new revised Manual of Clinical Microbiology, 7th edition, published by the American Society for Microbiology Press is an excellent reference for the identification of these and other gram positive organisms.

For information regarding submitting problem organisms call the reference bacteriology unit at 517-335-8134.

# Guide to Laboratory Services Now on Web!



The Bureau of Laboratories is happy to announce that the Guide to Laboratory Services is now available in a downloadable format on the DCH - BOL Web page. The guide was developed to assist users of the Bureau of Laboratories in the proper collection and submission of specimens for testing and in the interpretation of results. If you are unable to access this file, please contact Judith Smith at 517-335-8859 to request a copy.

www.mdch.state.mi.us/pha/bofl/Labguide/

### Congratulations!

John F. Riebow, Ph.D., has qualified for the Board of Certificate of Qualification from the American Board of Clinical Chemistry. Riebow, of the health risk assessment section, has successfully completed the professional experience component of the certification process. This component requires five years of full time and diverse experience in clinical chemistry.

Jim Armelagos is now a registered microbiologist with the American Society for Microbiology. Armelagos, a microbiologist in the quality assurance section, has a specialty registration in consumer and industrial microbiology with an emphasis on pharmaceuticals, medical devices and cosmetics.

### Penicillin Resistant Study-site<sup>1</sup> Isolates of Streptococcus pneumoniae and Vancomycin Resistant Sterile-site<sup>2</sup> Isolates of Enterococcus spp. Michigan Sentinel Hospital Laboratory Survey, Third Quarter, 1995 through Second Quarter, 1999

### Percent Resistant<sup>3</sup>

rters 1998 Quarters		1995 Quarters 1999 Quarters Third to Fourth		1996 Quarters First to Fourth		1997	1						
						First to Fourth		First to Fourth		First		Second	
roorganism	Resistance Classification <sup>3</sup>	Rg I	Rg 2-12	RgI	Rg 2-12	Rg I	Rg 2-12	Rg I	Rg 2-12	Rg I	Rg 2-12	Rg I	Rg 2-12
pneumoniae	Moderate or Hi	gh 20	14	25	18	24	22	21	23	25	21	19	27
pneumoniae	High Level only	5	4	7	3	11	5	5	7	9	4	7	10
recalis	Resistant	1	0	2	- 1	2	1	3	-1	3	0	2	1
recium	Resistant	34	7	41	9	49	9	56	40	68	24	69	34
Enterococcus	Resistant	8	1	10	2	13	4	14	7	17	6	18	8

dy sites = blood, CSF, deep surgical wound, pleural fluid(fl.), peritoneal fl., respiratory specimens or synovial fl. rile sites = blood, CSF, deep surgical wound, pleural fluid(fl.), peritoneal fl., or synovial fl. CCLS, Performance Standards for Antimicrobial Susceptibility Testing, M100 - S8.

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